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Enrichment of channel catfish (*Ictalurus punctatus*) fillets with conjugated linoleic acid and omega-3 fatty acids by dietary manipulation

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Abstract

An experiment was conducted in aquaria with channel catfish (*Ictalurus punctatus*) to determine the efficacy of augmenting fillets with conjugated linoleic acid (CLA) and omega-3 highly unsaturated fatty acids ($n-3$ HUFA) by feeding diets amended with products containing high levels of these nutrients. Refined menhaden fish oil at 1.5% of diet supplied the $n-3$ HUFA. CLA was used at dietary levels of 0.5% and 1% with a preparation that contained approximately 65% isomers of CLA. Corn oil was added to the basal diet at maximum inclusion level for added lipids of 3% for the control diet and to adjust total added lipid content of the other diets to 3%. Average initial body weight was 57.39±0.25 g/fish. Six experimental diets were fed twice daily to four replicate aquaria for six weeks. At that time, fish were group weighed for determination of weight gain and feed conversion. Fillets of six fish per aquarium were recovered and stored at −80 °C for moisture and total lipid analyses, fatty acid analysis, and sensory evaluation. Results showed feed consumption and feed conversion did not differ ($P>0.05$). Significantly ($P<0.05$) greater body weight gains were observed only for fish fed the diets with two combinations of CLA and 1.5% fish oil compared to fish fed the diet containing 0.5% CLA and corn oil. Fillet $n-3$ HUFA levels were significantly ($P<0.05$) elevated for fish fed diets containing fish oil when compared to $n-3$ HUFA of fillets of fish fed diets containing either corn oil or CLA and corn oil. Similarly, fillets from fish fed diets amended with CLA contained substantial amounts of CLA of up to 6.4% of total lipids. Fillets from fish fed corn oil or fish oil diets had no CLA. Taste panel evaluation indicated that fillets containing $n-3$ HUFA and CLA were essentially without fishy off-flavor and had excellent sensory qualities. Catfish fillets produced by amending diets with sources of $n-3$ HUFA and CLA at the levels used in this study would contain elevated levels of these nutraceuticals and could be an important human food source for these healthful fatty acids.

Keywords: Conjugated linoleic acid (CLA); Omega-3 highly unsaturated fatty acids; Channel catfish fillet; Nutraceutical; Functional food

1. Introduction

Conjugated linoleic acid (CLA) was identified as a component of dairy milk fat by Parodi (1977). Since that time there has been research to indicate that certain isomers that make up CLA have biological activity. Among the biological activities attributed to CLA include anticarcinogenic effect in rats (Ip et al., 1999), inhibition of atherosclerosis in rabbits (Lee et al., 1994), ability to alter lipid metabolism in certain animals such as swine (Ostrowska et al., 1999) and chickens (Badinga et al.,

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2003), and in humans (Gaullier et al., 2004). More recently, control of lipopolysaccharide-induced inflammatory diseases by one of the CLA isomers through down-regulation of the COX-2 protein has been reported (Li et al., 2005). A previous study with channel catfish showed that improvements in weight gain or alterations in percent carcass lipid content were not observed for fish fed diets supplemented with 0.5 or 1.0% CLA (Twibell and Wilson, 2003). Conjugated linoleic acid levels in muscle tissue were not determined in that study.

Presently, the main sources of CLA include food products derived from ruminating cattle or sheep. Products containing CLA include meat, milk, and cheese from these animals, but the typical levels found are very low e.g. approximately 0.5–1% of total fat in dairy milk (Hinders, 1999). With increased interest in foods containing nutraceuticals, which are natural compounds that have specific health benefits, manufacturers and producers of foods are developing new methods of delivering these substances to consumers. Industrial methods have been developed to prepare CLA from natural products such as safflower oil, which contains a high level (74%) of linoleic acid (NRC, 1993), the starting material for CLA. Conjugated linoleic acid is being used as a dietary supplement for humans, but the possibility exists that it could be added to the diets of food animals to increase the CLA content of meat produced from these animals.

Omega-3 highly unsaturated fatty acids ($n-3$ HUFA) have beneficial health effects attributable to the consumption of certain types of fish such as salmon, mackerel, tuna, and others. Among the apparent benefits cited for the consumption of foods containing $n-3$ HUFA are reductions in the occurrence of adverse cardiovascular events e.g. heart attack, cardiac arrhythmia, and thrombotic stroke (Kris-Etherton et al., 2002). Consumption of one of the $n-3$ HUFAs (docosahexaenoic acid, DHA) has been shown to be required for normal brain and eye development in infants (Horrocks and Yeo, 1999). Reduction of levels of beta amyloid plaque, the substance associated with Alzheimer's disease, was shown to occur in aged laboratory mice fed diets enriched with DHA (Lim et al., 2005). Previous studies in our laboratory have shown that fillets of channel catfish (*Ictalurus punctatus*) can be enriched with substantial amounts of $n-3$ HUFA by feeding diets that contained refined menhaden (*Brevoortia tyrannus*) fish oil (Manning et al., 2002). This fish oil contains high levels of the $n-3$ HUFA of interest such as eicosapentaenoic acid (EPA) and DHA.

This study was conducted to determine if channel catfish fingerlings fed diets amended with products that supplied CLA or $n-3$ HUFA singly or in combination could produce fillets containing substantial amounts of these beneficial fatty acids while still maintaining satisfactory sensory characteristics.

2. Materials and methods

Channel catfish fingerlings that had been maintained at the Thad Cochran National Warmwater Aquaculture Center, Stoneville, Mississippi, USA were randomly distributed into 24 100-l flowing-water aquaria at ten fish per aquarium. After two weeks of acclimation with a practical conditioning diet, the fish were consolidated and randomly re-distributed, ten fish per aquarium, among the 24 aquaria to achieve similar initial body weights. Average initial body weight was 57.39±0.25 g. Fish were maintained under conditions approved by the Mississippi State University Institutional Animal Use and Care Committee. Aquaria were equipped with air stones for aeration and water exchange was approximately 1 l/min. Dissolved oxygen levels were determined twice daily and maintained at 6.0±0.2 mg/l. Water temperatures were maintained at 30±2 °C. Aquaria were cleaned once a week.

Four aquaria of fish were randomly assigned to receive one of the six experimental diets and were fed weighed amounts of diet twice daily at 0800 h and 1600 h to apparent satiation based on percent body weight adjusted for feeding response. After six weeks, fish in each aquarium were group weighed and counted to determine average body weight and body weight gain. Feed consumption and feed conversion ratio (FCR) was determined for each group of fish. Six fish from each aquarium were randomly selected to provide fillets for moisture and total lipid analyses, gas chromatography (GC) analysis for fatty acids, and sensory evaluation by taste panel.

The experimental basal diet shown in Table 1 was formulated to provide all nutrients known to be required by channel catfish (NRC, 1993). Basal diet was amended with a total of 3% of three different lipids consisting of corn oil, refined menhaden oil, and the source of CLA. The lipid sources were added to the basal diet either singly or in combination as shown in Table 1 to provide six diets. Basal diet was prepared by blending all dry ingredients in a twin-shell V-mixer (Patterson-Kelly, East Stroudsburg, Pennsylvania) for 10 min. A weighed portion of the basal diet was transferred to a food mixer (Hobart Corporation, Troy, Ohio) and required amounts of the lipids were added. The mixture was blended thoroughly for 10 min. The complete diet mixture was moistened with deionized

Table 1
Composition of experimental diets containing combinations of conjugated linoleic acid (CLA) supplement, refined menhaden fish oil (FO), and corn oil (CO)

Ingredients	Basal diet (g/kg dry mixture)[a]
Soybean meal	505.0
Corn, cooked	200.0
Wheat middlings	120.0
Cottonseed meal	100.0
Carboxymethyl cellulose	30.0
Dicalcium phosphate	10.0
Vitamin premix[b]	2.5
Trace mineral mix[b]	1.0
Vitamin C[c]	1.5

Variable lipid components (g/kg)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
CO	30.0	15.0	7.5	–	22.5	15.0
FO	–	15.0	15.0	15.0	–	–
CLA supplement (65% CLA)[d]	–	–	7.5	15.0	7.5	15.0

[a]Calculated crude protein content of 32.04%.
[b]Catfish vitamin and trace mineral premixes were the same as described by Robinson and Li (1996).
[c]Vitamin C supplied by L-ascorbyl-2-polyphosphate (25% activity).
[d]CLA supplement contained by GC analysis the following CLA isomers: 18:2 cis9, trans11 (32.32%); −trans10, cis12 (27.92%); −cis9, cis11 (1.41%); −trans9, trans11 (2.07%).

water (425 g/kg) and mixed for 10 min in the mixer. Moistened diets were extruded with a food meat grinder (Hobart Corporation) and dried for 3 h in a forced-air oven at 25 °C to provide a dry matter content of 88–90%. Diets were prepared into suitable-size pellets with a circular food grinder; fines were removed and discarded using a sieve with 1.25 mm openings. Prepared diets were stored in sealed plastic bags at −18 °C until fed. Dry matter and percent total lipid content were determined in duplicate on 2-g samples of each diet (AOAC, 2000). Feed consumption was expressed on dry matter basis.

The two fillets from each of six fish from each aquarium collected at termination of the experiment were placed in individual, identified plastic storage bags and stored at −80 °C until needed for moisture and total lipid analyses, GC fatty acid (FA) analysis, or sensory panel evaluation. Preparation to conduct moisture, total lipid, and FA analyses consisted of grinding six fillets from different fish from each aquarium separately in a 1-l laboratory blender (Fisher Scientific, Pittsburgh, Pennsylvania) and storing separately in identified and sealed plastic bags at −80 °C. Analyses for moisture and total lipids were performed in duplicate on 2-g samples of a pooled sample of the six ground fillets from each aquarium (AOAC, 2000). The aquarium sample used for FA analysis consisted of a 2 g sample taken from each of the six ground fillets and ground together into a paste with a 35-ml capacity laboratory blender (Fisher Scientific, Pittsburgh, Pennsylvania). Fat was extracted from a 1-g subsample by a modified chloroform–methanol method of Folch et al. (1957). After a series of liquid/liquid phase separations, centrifugation, and evaporation under nitrogen, the lipid fraction was methylated with boron trifluoromethanol (Sigma Chemical Co. St. Louis, MO). Fatty acid methyl esters (FAME) were determined by a modified American Oil Chemists Society (AOCS)-AOAC method (AOAC, 1998) with a GC (HP 5890 Series II, HP 3365 Chem-Station; Hewlett-Packard Co. Avondale, Pennsylvania) equipped with a DB-Wax capillary column (30 m, 0.32 mm i.d., 0.25 cm film thickness) (J&W Scientific Co., Agilent Technologies, Wilmington, Delaware) and a flame ionization detector. Identification of FAME was based on comparisons of GC analyses of authentic FAME standards (NU-Chek-Prep, Elysian, Minnesota). One fillet FA analysis was determined for each replicate aquarium of fish. Additionally, fatty acid analysis of a 1-g sample of each diet was completed by the same method described above for FA analysis of fish fillets.

Sensory evaluation was conducted with a panel consisting of four participants who were trained to taste catfish fillets. Sensory evaluation was conducted under guidelines established by Mississippi State University's Institutional Review Board. Three fillets from different fish of each aquarium were ground in a kitchen food processor. The three ground fillets were blended and hand-formed into patties that were cooked for 70 s in a 1000-W microwave oven and immediately presented to the panelists. Fillet samples were randomized and identified by a numerical code that was unknown to panel members. Evaluation consisted of determination of taste preference and the presence of fishy off-flavors or other identifiable, undesirable sensory characteristics. A numerical scoring system was used to indicate degree of fishy flavor determined for a fillet sample. The scores consisted of numerical values ranging from zero (0) to four (4) with zero indicating that no fishy flavor was detected and four indicating that the fillet sample had distinctly fishy flavor. Each panel member recorded on an individual score sheet the numerical value that best described the level of fishy flavor of the tasted fillet sample. Scores of each fillet sample were summarized among panel participants.

Data for weight gain, feed consumption, feed conversion, total lipid content, and levels of CLA and

Table 2
Selected fatty acid content as determined by GC analysis of experimental diets amended with conjugated linoleic acid (CLA), refined menhaden fish oil (FO), and corn oil (CO)[a]

Diet description[b]	Fatty acid content (% of total lipids)[c, d]							
	18:2*n*−6	18:3*n*−3	c9,t11	t10,c12	c9,c11	t9,t11	20:5*n*−3	22:6*n*−3
CO, 3%	47.70	2.77	–	–	–	–	–	–
FO, 1.5%; CO, 1.5%	36.95	2.66	–	–	–	–	3.23	3.16
CLA, 0.5%; FO, 1.5%; CO, 0.75%	29.30	2.52	6.54	5.64	0.13	1.97	1.95	1.56
CLA, 1.0%; FO, 1.5%	21.49	2.16	10.28	9.46	0.42	3.73	2.11	1.86
CLA, 0.5%; CO, 2.25%	37.94	2.28	5.07	4.86	0.22	2.07	–	–
CLA, 1.0%; CO, 1.5%	33.98	2.16	8.34	8.16	0.44	4.01	–	–

[a]All diets were amended with a combined total of 3.0% lipids consisting of corn oil, refined menhaden fish oil, and/or CLA supplement containing approximately 65% CLA isomers.
[b]Diets containing 0.5% or 1.0% CLA were amended with 0.75% or 1.5% CLA supplement, respectively, to adjust for the approximately 65% isomer content.
[c]Fatty acids are as follows: linoleic acid, 18:2*n*−6; linolenic acid, 18:3*n*−3; CLA isomers, 18:2 (−cis9, trans11) (−trans10, cis12) (−cis9, cis11) (−trans9, trans11); 20:5*n*−3, eicosapentaenoic acid; 22:6*n*−3, docosahexaenoic acid.
[d]One GC fatty acid determination for each diet.

n−3 HUFA in fillets were analyzed by ANOVA using the general linear model (GLM). Duncan's multiple-range test was used to determine significant differences among treatment means at $P<0.05$. SAS ver. 8.0 (SAS Institute, Inc. Cary, North Carolina 2000) and Steel et al. (1997) were used for statistical analysis.

3. Results

Selected fatty acids (including CLA isomers and *n*−3 HUFA) in the experimental diets are shown in Table 2. Diets contained sufficient levels of the expected fatty acids based on lipid supplementation. The two predominant CLA isomers determined to be present in the CLA supplemented diets were 18:2 c9,t11 and 18:2 t10,c12. Fish performance data showed that no significant ($P>0.05$) differences were observed among treatments for feed consumption and feed conversion (Table 3). Body weight gain of fish fed the diet containing 0.5% CLA isomers and 2.25% corn oil was significantly ($P<0.05$) lower than weight gain of the two other dietary treatments containing the combinations of CLA at 0.5% or 1.0% with 1.5% menhaden fish oil. Incorporation of the CLA supplement into the basal diet at 1.0% CLA isomers in combination of with 1.5% refined menhaden fish oil significantly ($P<0.05$) elevated the CLA content of fillets compared to fillets from catfish fed the diet containing 1.0% CLA isomers in combination with 1.5% corn oil (Table 4). A similar but non-significant ($P>0.05$) trend was observed for fillets from fish fed the diet supplemented with 0.5% CLA isomers, 0.75% corn oil and 1.5% fish oil when compared with fillets from the diet containing 0.5% CLA isomers and 2.25% corn oil. Fillet CLA content significantly ($P<0.05$) increased in response to increased dietary levels of the CLA supplement. No CLA was detected by GC analysis of fillets of fish fed

Table 3
Body weight gain, feed consumption, and feed conversion of channel catfish fed practical diets amended with combinations of corn oil (CO), refined menhaden fish oil (FO), and conjugated linoleic acid (CLA)

Diet description[a]	Body weight gain (g/fish)	Feed consumption (g/fish)	Feed conversion (g feed/g gain)
CO, 3%	149.83^{z,y}	182.43	1.22
FO, 1.5%; CO, 1.5%	141.43^{z,y}	182.05	1.29
CLA, 0.5% (0.75); FO, 1.5%; CO, 0.75%	154.26^{z}	182.16	1.18
CLA, 1.0% (1.5); FO, 1.5%	154.22^{z}	182.31	1.18
CLA, 0.5% (0.75); CO, 2.25%	134.80^{y}	182.02	1.38
CLA, 1.0% (1.5); CO, 1.5%	138.63^{z,y}	182.44	1.32
Pooled SEM	4.97	0.79	0.56
P value	0.047	0.998	0.115

[a]Values in parentheses are the percent added CLA supplement containing approximately 65% isomers needed to achieve 0.5% or 1.0% dietary CLA. Means in a column with uncommon superscript are significantly different ($P<0.05$).

Table 4
Conjugated linoleic acid (CLA) and omega-3 highly unsaturated fatty acids ($n-3$ HUFA) content of fillets from channel catfish fed diets amended with corn oil (CO), refined menhaden fish oil (FO), and CLA supplement for 6 weeks

Diet description[a]	CLA[b] (% of total lipid content)	Omega-3 HUFA[c] (% of total lipid content)	Total lipid content[d] (%)
CO, 3%	–	1.52[y]	6.58
FO, 1.5%; CO, 1.5%	–	4.94[z]	6.06
CLA, 0.5% (0.75); FO, 1.5%; CO, 0.75%	3.58[x]	5.20[z]	5.58
CLA, 1.0% (1.5); FO, 1.5%	6.40[z]	5.39[z]	5.61
CLA, 0.5% (0.75); CO, 2.25%	3.26[x]	2.42[y]	5.55
CLA, 1.0% (1.5); CO, 1.5%	5.85[y]	1.84[y]	5.64
Pooled SEM	0.13	0.37	0.25
P value	0.0001	0.0001	0.056

[a]Values in parentheses are percent added CLA supplement containing approximately 65% isomers needed achieve 0.5% and 1.0% dietary CLA. See Table 2 footnote [b] for additional explanation.
[b]Total CLA content based on GC analysis of six pooled fillets from different fish of each aquarium that included CLA 18:2 c9,t11; −t10,c12; −c9,c11; and −t9,t11 isomers. One GC determination per aquarium; mean of four determinations per dietary treatment.
[c]Total omega-3 HUFA content based on GC analysis of six pooled fillets from different fish of each aquarium that included eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). One GC determination per aquarium; mean of four determinations per diet.
[d]Individual aquarium values were determined in duplicate from weighed 2-g samples of a pooled sample of six fillets. Tabular values are means of determinations for four replicate aquaria.
Means within a column with uncommon superscript are significantly different ($P<0.05$).

the two diets containing 3% corn oil or 1.5% corn oil and 1.5% fish oil. Feeding diets containing 1.5% fish oil (with 1.5% corn oil) or the combination of 1.5% fish oil and the two levels of CLA produced fillets with significantly ($P<0.05$) elevated levels of $n-3$ HUFA when compared with fillets from fish fed diets containing 3% corn oil or the two combinations of CLA and corn oil. There were no significant differences among treatments for fillet total lipid content as shown in Table 4. Sensory panel results indicated that of a total of 96 evaluations among the four participants there were only 4 (4.2%) evaluations that indicated the presence of a very slightly fishy taste. Generally, all samples of the fillets were favorably accepted by the panelists.

4. Discussion

The results of this experiment demonstrate that channel catfish fingerlings fed diets amended with a CLA supplement and/or refined menhaden fish oil for six weeks can accumulate significant quantities of CLA and omega-3 HUFA in muscle tissue. Twibell et al. (2001) found that levels of these two types of fatty acids were significantly increased in the muscle tissue of yellow perch (*Perca flavescens*) fed diets containing 0.5% or 1.0% CLA and 8.0% menhaden oil. Similarly, Berge et al. (2004) found that feeding diets containing 0, 0.5, 1.0, or 2.0% CLA significantly increased the levels of CLA in tissues of juvenile Atlantic salmon (*Salmo salar*) in amounts that were closely correlated with the dietary concentrations of CLA.

For the present study, accumulation of these two important groups of fatty acids in catfish fillets resulted in fillets that had satisfactory flavor characteristics according to taste panel participants. Taste of fillets from fish fed the diets amended with CLA and refined menhaden fish oil was similar to the taste of fillets from fish fed the corn oil diet. While the benefits of consuming foods that provide appreciable amounts of omega-3 HUFA have been cited many times, the benefits of consuming food products containing CLA are less well known, but of equal importance. Until recently, only foods that were derived from ruminant animal production contained CLA. Development of manufacturing processes that convert vegetable oil such as safflower oil, which is high in linoleic acid, to CLA makes available a CLA supplement that could be used in animal feeds. Feeds that contain CLA could be used to add CLA to animal food products such as channel catfish that traditionally do not contain these fatty acids.

No attempt was made in this study to estimate the cost of producing CLA enriched catfish fillets, because the availability of CLA supplements is limited, especially for feed manufacturing purposes. As availability increases, the cost of CLA supplements for catfish feeds should decline. For a dairy product such as whole milk containing about 3.7% total fat of which approximately 1.1% is CLA (Hinders, 1999), a modest-sized serving of milk of approximately 150 g would provide about 61 mg CLA. In contrast, a 150 g serving of catfish fillet having similar lipid characteristics that were demonstrated for fish fed the diet containing 1.0% CLA in combination

with 1.5% fish oil would provide over 500 mg CLA and 450 mg omega-3 HUFA.

5. Conclusion

Functional foods that provide beneficial, health-promoting components are attracting increasingly more attention over the past few years. Channel catfish fillets enriched with CLA and omega-3 HUFA could contribute ample amounts of these fatty acids to the diets of people. Moreover, producing a food such as fillets enriched with CLA and $n-3$ HUFA may offer a unique opportunity to catfish producers and processors to develop a new food product.

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